

# Cross-talk between pyrene & hypoxia signaling pathways in embryonic *Cyprinodon variegatus*



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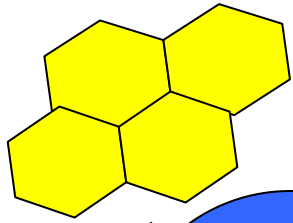
Gulf Coast Research Laboratory - Ocean Springs, MS



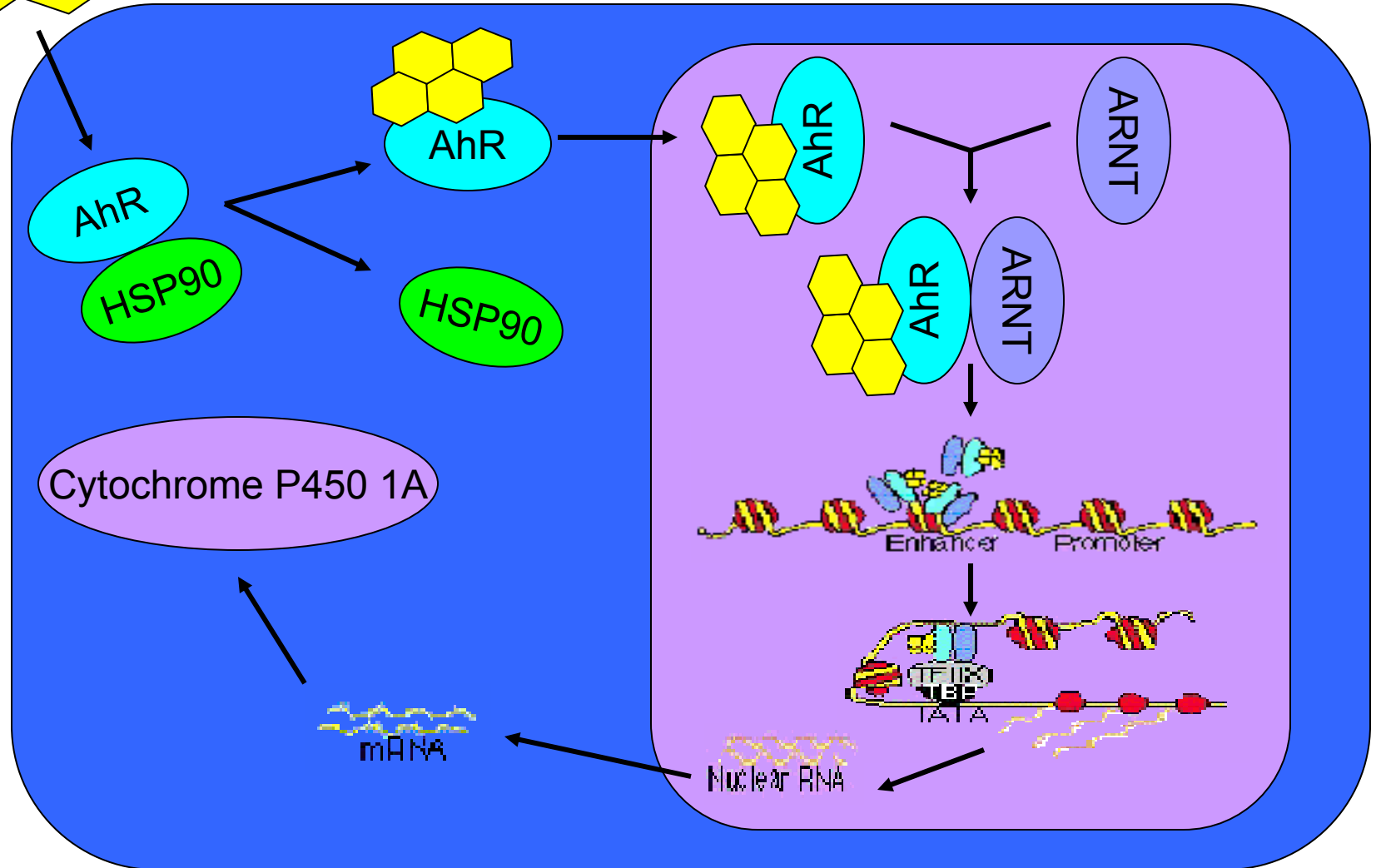
# Polycyclic Aromatic Hydrocarbons (PAHs)

- ◆ Enter water column & sediments
  - Dust and precipitation
  - Discharges from industrial/wastewater treatment plants
  - Land-based runoff
- ◆ Near shore areas
  - Spawning grounds
  - Embryo development susceptible to PAHs

Pyrene

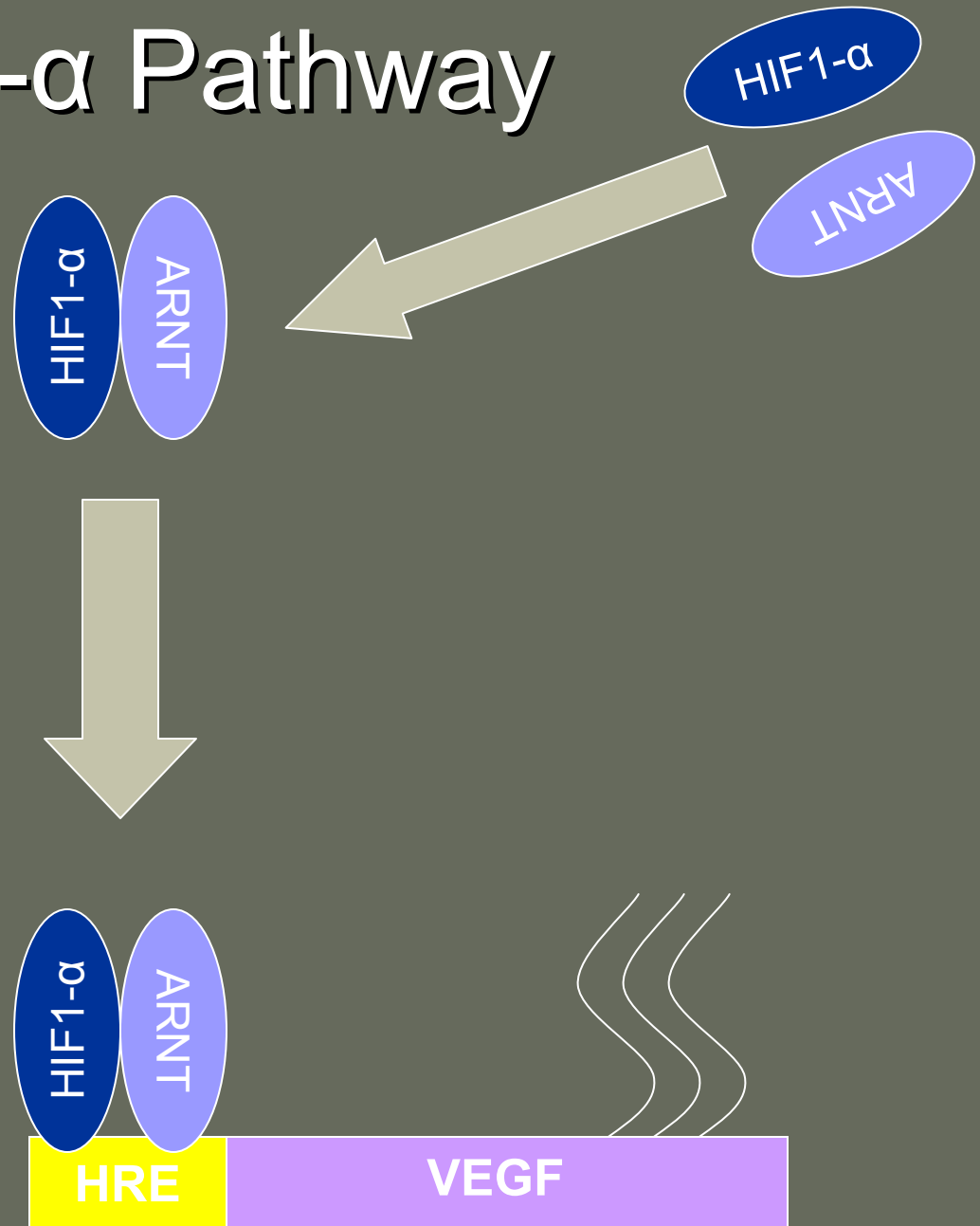


# Signaling Pathway





# HIF1- $\alpha$ Pathway



# Objective

- ◆ Determine if pyrene activation of AhR2 or hypoxia-activation of HIF1- $\alpha$  results in inhibition of the other, resulting in reduced developmental toxicity associated with pyrene or hypoxia alone in embryonic *Cyprinodon variegatus*

# Laboratory Exposures

- ◆ Study 1 – Hypoxia Exposure
  - 6–8 ppm
  - 1–2 ppm
- ◆ Study 2 – Pyrene Exposure
  - 0, 20, 60, 150 ppb
- ◆ Study 3 – Combined Exposure
  - 6–8 ppm
  - 1–2 ppm
  - 0, 20, 60, 150 ppb

# Goals

- ◆ Determine developmental toxicity endpoints (pre- & post-hatch)
  - delayed development
  - curvature
  - pericardial / yolk-sac edema
- ◆ Use q RT-PCR to measure expression levels at 12, 24, 48, 96, & 432 hpf
  - CYP1A1
  - VEGF
    - ◆ Cloned CYP1A1, VEGF, & 18S rRNA partial nucleotide sequences
    - ◆ Designed gene-specific primers



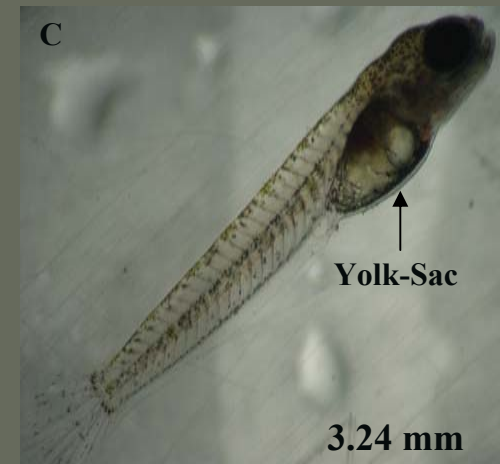
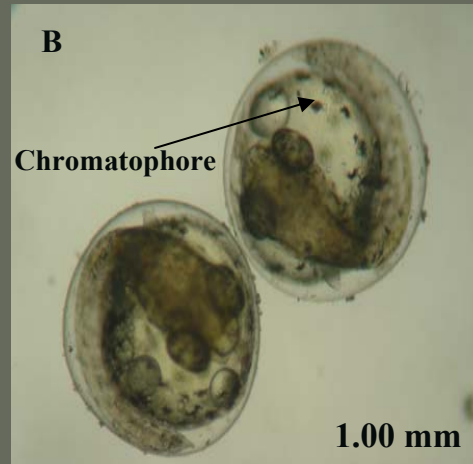
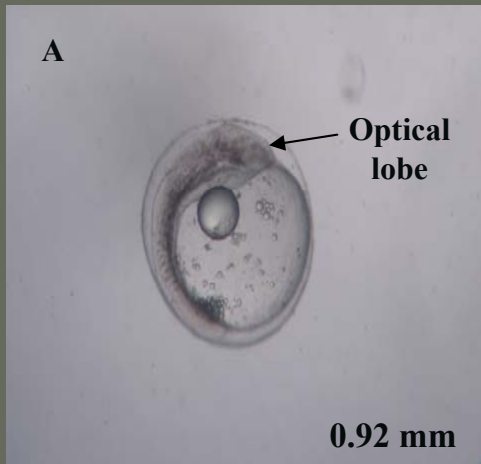
# Study 1 – Hypoxic Embryonic Development

24 hpf

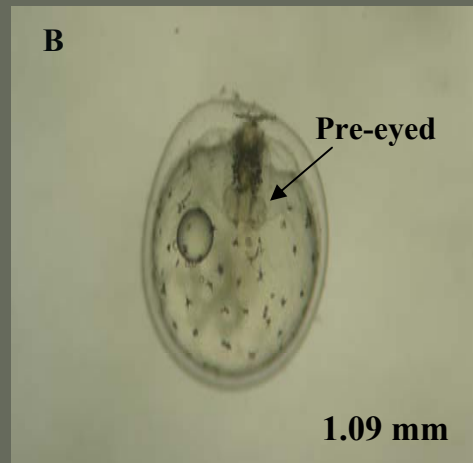
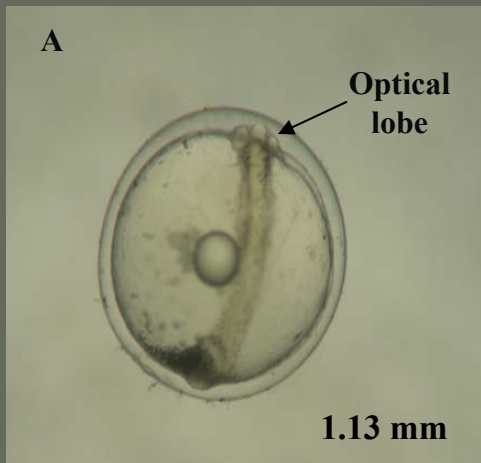
48 hpf

120 hpf

Normoxia



Hypoxia



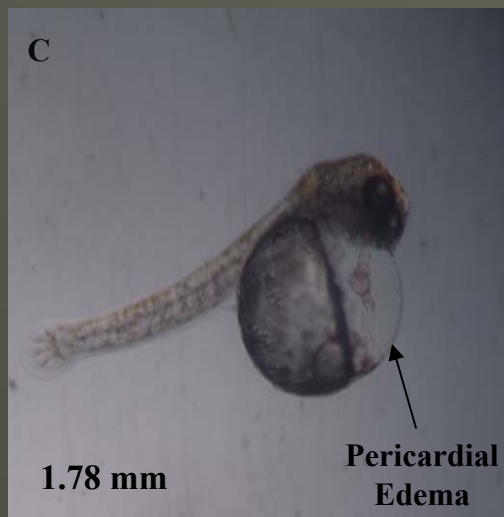
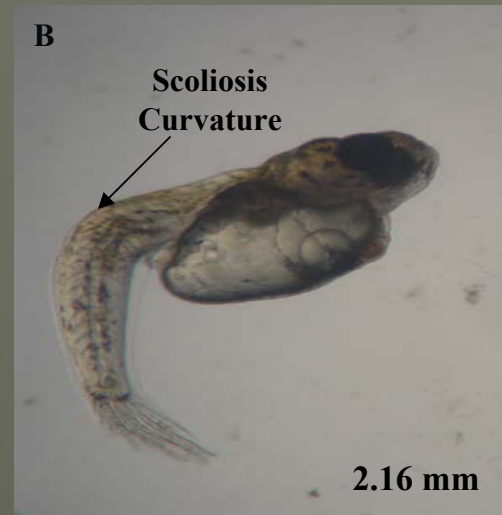
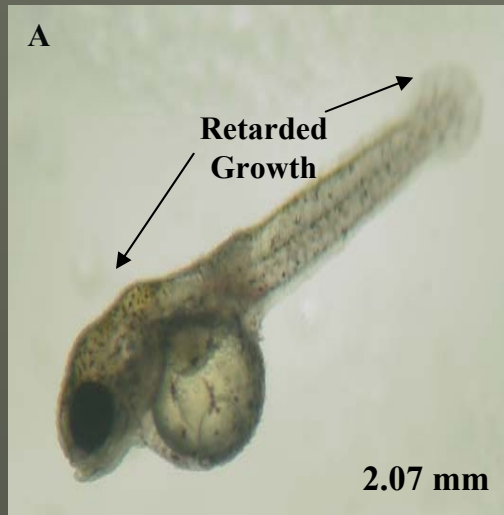


# Study 1 – Hypoxia Exposure

	<b>Days to Hatch (dpf)</b>	<b>Total Hatch</b>	<b>Total Survival (18 dpf)</b>
<b>Normoxia</b>	5	100 %	98 %
<b>Hypoxia</b>	11	62 % *	37 % *

\* Indicates significance between control, where  $p \leq 0.05$

# Study 1 - Hypoxia-Exposed Fry



# Study 1 – CYP1A1 & VEGF Expression

	Dose-Response	Time-Response
<b>CYP1A1</b>	nd (12 hpf) – (24-96 hpf) ↑ (432 hpf)	nd (12 hpf) ↑ (all)
<b>VEGF</b>	– (12,48,96 hpf) ↓ (24 hpf) ↑ (432 hpf)	↑ (normoxia) – (hypoxia)

nd = not detected

# Conclusions

- ◆ Hypoxia causes long-term responses in the overall stress of *C. variegatus*
  - Delayed development / hatching
  - Significant mortality
  - Retarded growth
  - Curvature
- ◆ VEGF involved in normal development
- ◆ Hypoxia appears to halt VEGF expression in embryos
- ◆ CYP1A1 unaffected by hypoxic conditions

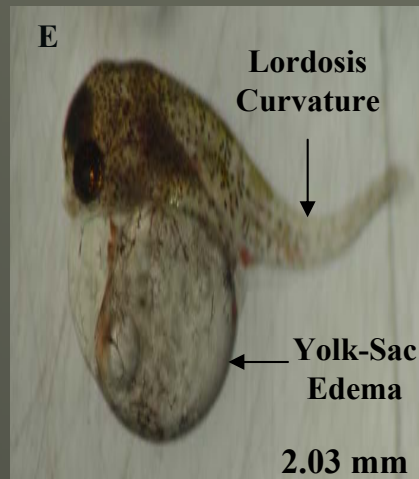
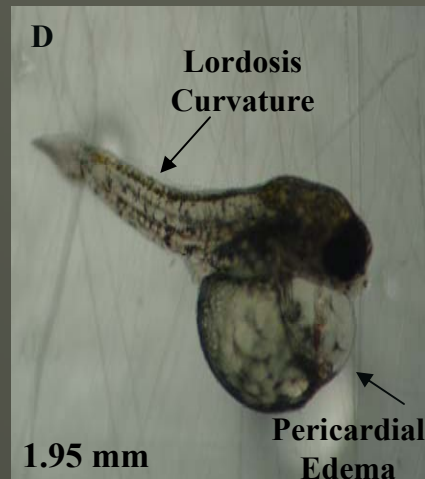
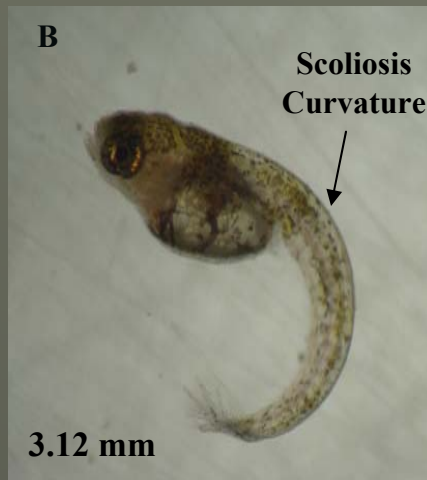
# Study 2 – Pyrene Exposure

	<b>Days to Hatch (dpf)</b>	<b>Total Hatch</b>	<b>Total Survival (18 dpf)</b>	<b>Standard Length (mm)</b>
<b>CTL</b>	5	100 %	93 %	5.93±0.07
<b>DMSO</b>	5	98 %	88 %	5.87±0.13
<b>20 ppb</b>	5	98 %	80 %	5.09±0.12 *
<b>60 ppb</b>	5	98 %	9 % *	m
<b>150 ppb</b>	5	61 % *	m	m

m = 100 % mortality before conclusion of exposure

\* Indicates significance from all remaining treatments, where  $p \leq 0.05$  as determined by ANOVA

# Study 2 - Pyrene-Exposed Fry



**A = 60 ppb**

**B-E = 150 ppb**



# Study 2- CYP1A1 & VEGF Expression

	Dose-Response	Time-Response
CYP1A1	nd (12 hpf) ↑ (all)	nd (12 hpf) ↑ (all)
VEGF	— (all)	↑ (all)

nd = not detected

# Conclusions

- ◆ Pyrene caused toxicity in fry
  - pericardial/yolk edema
  - dorsal curvature
  - reduced growth
  - significant mortality
- ◆ CYP1A1 is a pyrene-inducible gene
  - Dose-dependent
  - Time-dependent
- ◆ VEGF is independent of pyrene dosage
  - Time-dependent

# Study 3 – Combined Exposure

	<b>Days to Hatch (dpf)</b>	<b>Total Hatch</b>	<b>Total Survival (18 dpf)</b>
<b>CTL – Norm</b>	5	100 %	99 %
<b>CTL – Hyp</b>	12	55 % *	49 % *
<b>20 ppb – Hyp</b>	14	2 % **	17 % **
<b>60 ppb – Hyp</b>	-	0 %	m
<b>150 ppb – Hyp</b>	-	0 %	m

m = 100 % mortality before conclusion of exposure

- = 0 % hatch success exposure

\* Indicates significance from CTL-Norm

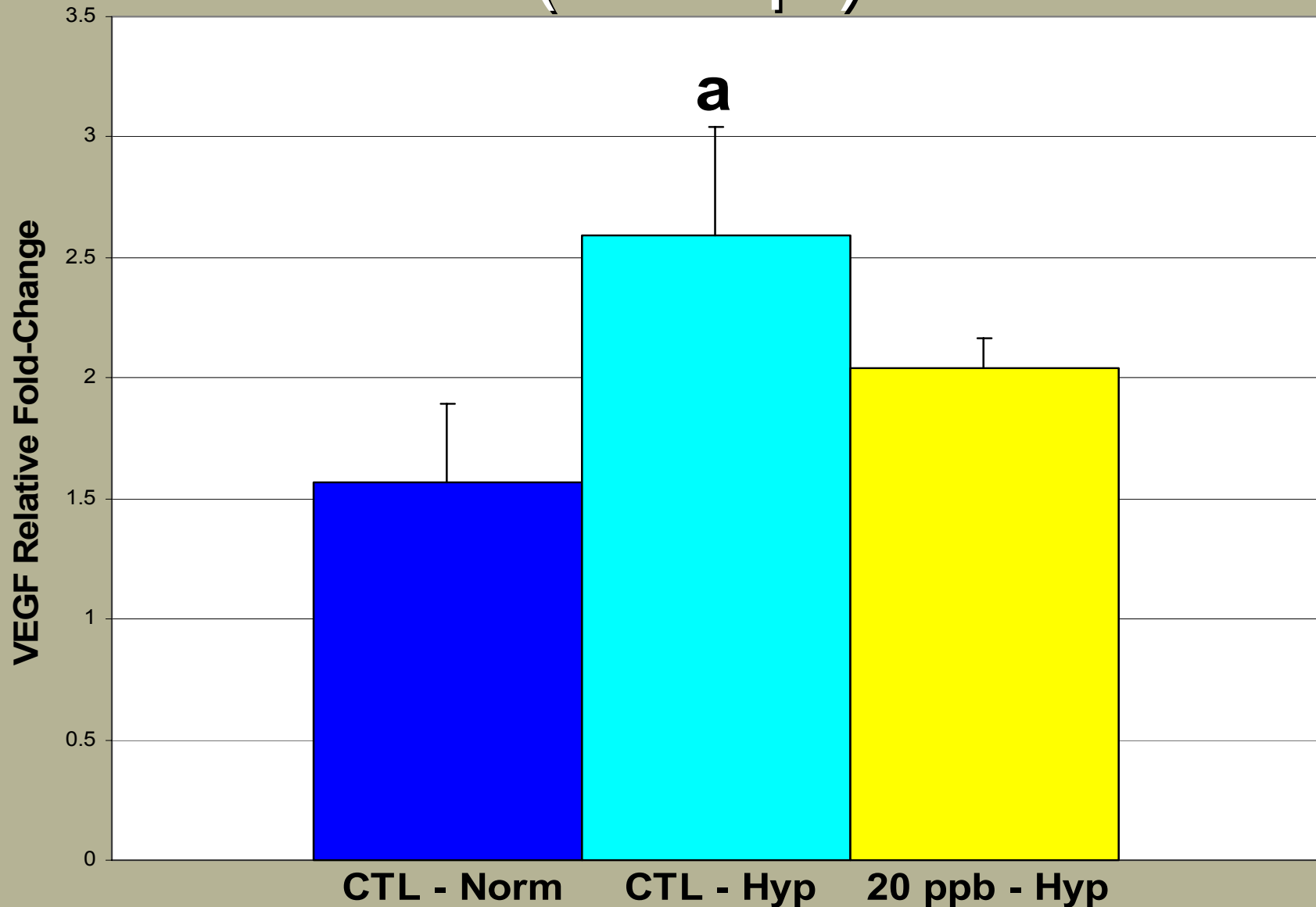
\*\* Indicates significance from both controls

# Study 3 – CYP1A1 & VEGF Expression

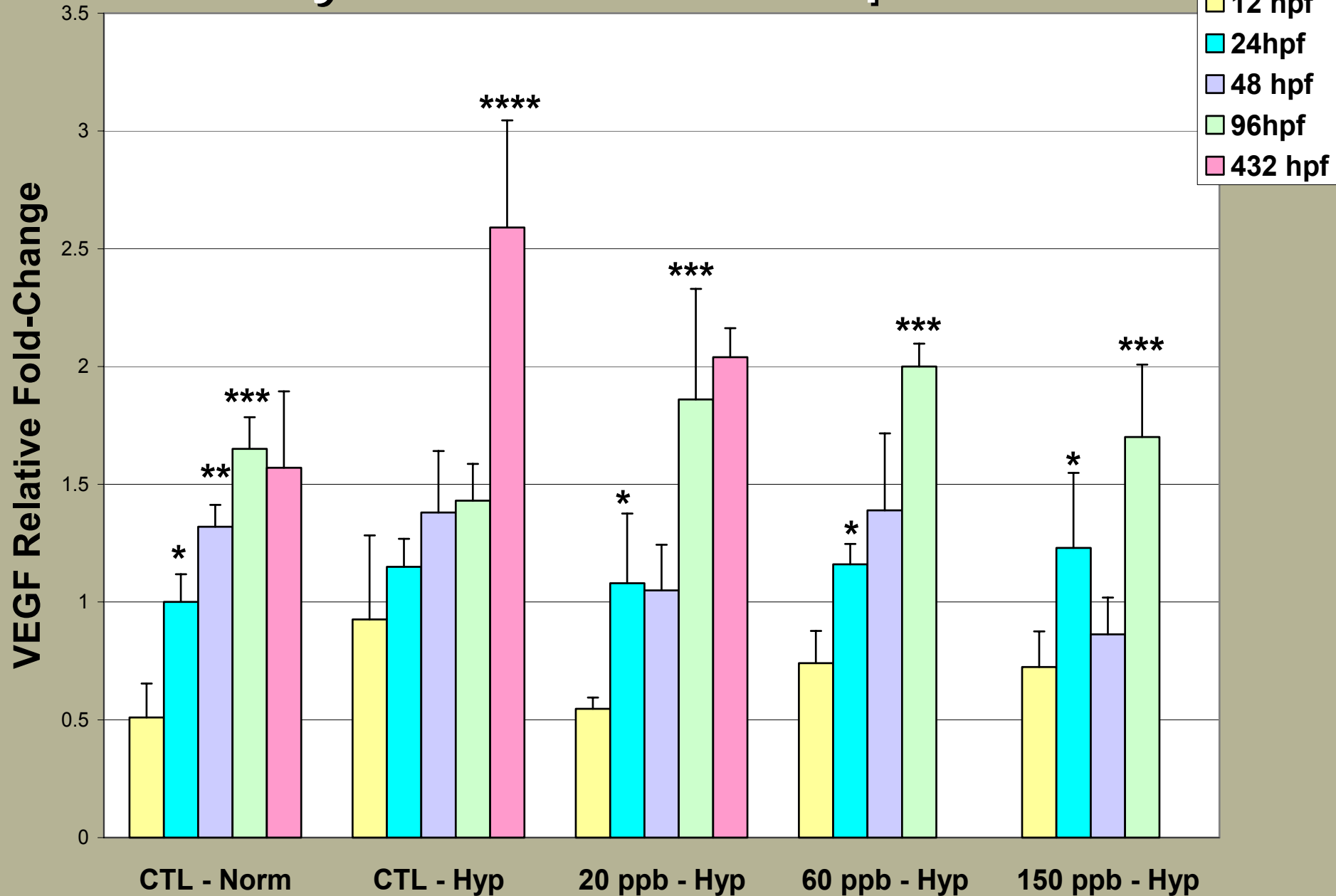
	Dose-Response	Time-Response
CYP1A1	nd (12 hpf) ↑ (all)	nd (12 hpf) ↑ (all)
VEGF	– (12-96 hpf) ↑ (432, CTL-Hyp) – (432, 20ppb-Hyp)	↑ (CTL-Norm) – (CTL-Hyp) ↑ (Pyrene-Hyp)

nd = not detected

# Study 3 – VEGF Expression (432 hpf)



# Study 3 – VEGF Expression





# Conclusions

- ◆ Combined exposure results in severe embryonic toxicity
  - No hatching
  - Significant mortality
- ◆ CYP1A1 regulation unaffected by hypoxia
  - Significantly up-regulated
    - ◆ Dose-dependent
    - ◆ Time-dependent
- ◆ VEGF expression altered by hypoxia but NOT pyrene
  - NOT Dose Dependent
  - Time-Dependent
    - ◆ Significantly up-regulated

# Cross-Talk?

- ◆ Still Unclear . . .
- ◆ HIF1- $\alpha$  levels NOT measured
  - Negative regulator of HIF1- $\alpha$  may be induced
    - ◆ Prolyl Hydroxylase-3
- ◆ Inhibition of HIF1- $\alpha$  pathway by combined doses of pyrene & hypoxia

# Acknowledgements

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